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TITLE: Estrogen Receptor Gene Polymorphisms and Breast Cancer Risk

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13. ABSTRACT ***(Maximum 200 Words)***

Purpose: *to test the hypothesis that individual variation in the sensitivity to estrogens, whether from endogenous or exogenous sources, affects woman's risk of breast cancer.*

Scope: *to test if the variability in responses to estrogens in women may be determined by a functionally significant variation in the estrogen receptor alpha (ER) though which estrogens exert biologic effects. The ER polymorphisms under study are: Xba I, Pvu II and microsatelite polymorphisms. In an exploratory analysis we will study the interaction between the Xba I, Pvu II and microsatellite ER polymorphisms and estrogen-related risk factors for breast cancer such as reproductive history and menopausal hormone use.*

Major findings: *not yet available (biological sample collection is still ongoing)*

Up-to-date report of the progress in terms of results and significance: *no results are yet available – we are still collecting biological samples, extracting DNA from the biological materials (leukocytes and pathological slides) and processing the laboratory analysis. These activities are following the time schedule as stated in the Proposal to the Army. We expect to have preliminary results by the end of year 2000.*

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FOREWORD

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N/A In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

X For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

N/A In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

N/A In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

N/A In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

PI - Signature Date

(4) TABLE OF CONTENTS (First annual report – grant DAMD17-97-1-7322)

(5) INTRODUCTION:

***Subject*:**

The research being carried out aims to clarify how estrogens interact with the estrogen receptor alpha in the causation of breast cancer and to define women at high risk of developing this malignancy.

Purpose:

The hypotheses to be tested in this study are:

1) There is an association between the Xba I and Pvu II polymorphisms in the estrogen receptor alpha gene and the risk of breast cancer;
2) There is an association between microsatellite polymorphisms in the estrogen receptor gene and the risk of breast cancer.

An exploratory hypothesis to be considered in the research proposed is:

3) There will be an interaction between the Xba I, Pvu II and microsatelitte ER alpha polymorphisms and estrogen-related risk factors for breast cancer such as reproductive history and menopausal hormone use.

Scope (i.e. Specific Tasks, as outlined in the approved Statement of Work approved by the US Army) :

1) To obtain blood samples from 600 breast cancer cases and 600 population based controls.

2) NOTE: this sample size will be complemented with an additional 1200 breast cancer cases and 1200 population controls samples. The additional breast cancer cases and controls subjects are sponsored by the NIH, as agreed with the US Army – please see further explanation below. Overall, at least 300 cases and 300 controls will never be exposed to menopausal hormone use, and at least 300 cases and 300 controls will have been users of postmenopausal hormones for at least 4 years;

3) To extract DNA from white blood cells (or from normal tissue cells, obtained from freshly cut surgical blocks, when breast cancer cases prefer not to donate a blood sample but consent and give permission to use their surgical materials);

4) To assess microsatellite, Xba I and Pvu II germline polymorphisms in the estrogen receptor gene in the cases and controls

5) To assess the relative risk for breast cancer associated with the microsatellite, Xba I and Pvu II polymorphisms, and to explore interactions between the polymorphisms and estrogen related breast cancer risk factors.

(6) BODY:

In this section we describe the research accomplishment associated with each Specific Task (Scope) outlined in the approved Statement of Work.

We present the available data - i.e. results from the recruitment period - as comprehensively as we can.

We can not report now on findings based on the laboratory analysis, since we are still enrolling subjects into the study. Results on the laboratory analysis of gene polymorphisms will be provided on our next report, due to December 2000.

This study has been funded simultaneously by the NIH and by the US Army. An agreement with the US Army was reached at the beginning of 1999, to avoid overlapping of resources. In this agreement we committed ourselves in increasing the number of breasts cancer cases and control subjects enrolled in our study.

We will describe accomplishment associated with each Task (i.e. Scope):

Scope 1 and 2 (specific tasks 1 and 2):

The Table below summarizes the progress on subjects recruitment from May 1998 to November 1999.

'Selection 1' corresponds to the part of the study that is supported by NIH, where approximately 1200 breast cancer cases and 1200 control subjects would be recruited.

'Selection 2' corresponds to the part of the study that is exclusively supported by the US Army. The recruitment of subjects described in 'Selection 1' is more complete than from 'Selection 2' because the contract negotiations with the US Army were delayed. Therefore we waited to start recruiting subjects until we finalized the agreement with the Army (which took place in March1999; our contact person for more information about this issue is Cheryl R Miles at the US Army). We expect participation rates for 'Selection 2 to be approximately similar to those in 'Selection 1'.

Table 1. Recruiting of study subjects (May 1998 to November 1999) *

	Selection 1		Selection 2	
	Cases	Controls	Cases	Controls
Selected no	1198	1112	600	452 [(a)]
Information sent	1056	1072	251	224
Deceased, total no [(b)]	171 (14.3%)	55 (4.9%)	99 (16.5%)	19 (4.2%)
Response [(c)]	1039 (86.7%)	1047 (94.2%)	168 (66.9% [d])	146 (65.2% [d])
Blood sample	918 (76.6% [e])	854 (76.8% [e])	152 (60.6% [d])	95 (42.4% [d])
Tissue sample	232 (19.3%)	Not applicable	11	Not applicable
Decline to participate	31 (2.6%)	228 (20.5%)	5 (2.0%)	51 (22.8%)
No decision	17	27	- [(g)]	- [(g)]
Blood sample arrived [(f)]	907 (98.8%)	835 (97.8%)	95 (62.5%)	53 (55.8%)
Participation, total	95.9%	76.8%	- [(g)]	- [(g)]

* IMPORTANT: please note that we have just start working with women from 'Selection 2'. We will complete the mailing of information to women from 'Selection 2' in the forthcoming weeks. The total participation rate from women in 'Selection 2' is expected to be at least as high as among women from 'Selection 1'.

(a) 600 controls were selected but 148 have been contacted and asked to participate in another study, 79.5% of those 148 have agreed to donate a blood sample
(b) Of all selected
(c) Includes diseased cases from whom we intend to use the archived tissue sample.
(d) Of those to whom information has been sent
(e) Of all selected
(f) Of those who have agreed to donate a blood sample
(g) Subjects are currently being contacted

Current work:

Selection 1 (the component of the study partly funded by the NIH).

We Attempt to reach subjects who have not responded to our invitation to participate in the study. We also intend to contact by phone subjects who have agreed to donate a blood sample but still have not done so.
Some women denied a blood sample, but allowed us to use their stored surgical material for our research. We are now contacting the pathology departments in Sweden to obtain freshly cut tissue, from which we extract DNA and process the analysis proposed in this study.

Selection 2. (the component of the study funded solely by the US Army)

We are now sending information to the selected study subjects and asking for consent to participate. Collection of blood samples has just started. Collection of stored biological material will be performed for those breast cancer patients who disagree in donating a blood sample but agree in allowing the use of their surgical material.

Scope 3 (specific task 3):

- Samples are continuously being transferred to the laboratories for DNA isolation and analysis of the polymorphisms.
- DNA from nearly all blood samples from *Selection 1* have been isolated.
- DNA from blood samples from Selection 2 has started to be isolated. He hope to finalize all the analyses on the fall of the year 2000.

Scope 4 (specific task 4):

- Not yet performed.
- The statistical analysis will be performed when all samples - i.e. from *Selection 1* and *Selection 2* - have been collected and analyzed in the laboratory.

Problems in accomplishing any of the tasks:

- The DNA originating from tissue samples (surgical material) was too fragmented for the XbaI and PvuII RFLP analyses to be performed.
- The restriction sites have therefore been *successfully sequenced* in order to be able to analyze the polymorphisms using the minisequencing single nucleotide primer extension assay.
- The method has been tested and validated and the genotyping is ongoing.

Findings (positive or negative): Not yet available.

Publications based on this study: Not yet available.

Presentations based on this study: Not yet performed.

Statistical tests of significance shall be applied to all data whenever possible: Does not apply (yet).

Figures and graphs: Not available yet

Discussion (including relevance of the original hypothesis): Does not apply yet.

Recommended changes or future work to better address the research topic: None available yet.

(7) KEY RESEARCH ACCOMPLISHMENTS

- Results from this research are not yet available.
- We are still collecting biological material and performing laboratory work.
- Preliminary results are expected to be available by the end of year 2000.

(8) REPORTABLE OUTCOMES:

- *manuscripts, abstracts, presentations*

  Not yet available.

- *patents and licenses applied for and/or issued:*

  Not applicable.

- *degrees obtained that are supported by this award:*

  Two cancer epidemiologists in training are working on this project, namely:

  As a post-doctoral fellow: Elisabete Weiderpass, M.D.,Ph.D.
  As a Ph.D. student: Sara Wedren, M.D.

- *Development of cell lines, tissue or serum repositories:*

DNA from breast cancer patients and control subjects are being extracted from white blood cells and stored in the collaborating laboratories at Uppsala University.

The storage and use of this material follows the Swedish laws of safeguarding of privacy, and has been approved by the local Ethics Committees.

- Informatics such as databases and animal models, etc:

  Not applicable.

- Funding applied for based on work supported by this award:

  Not applicable

- Employment or research opportunities applied for and/or received on experiences/training supported by this award:

  None at the moment.

(9) CONCLUSIONS:

- Currently we do not have any laboratory results available for the whole material (we are still collecting biological material and performing laboratory analyses).

- Laboratory personnel are blinded (unaware) to the case-control status of each sample. The results from the laboratory work have not yet been linked to the database containing information on case-control status. Therefore we can not present any conclusions or preliminary conclusions.

- Results will be available by the end of year 2000 (an extension of the period to submit the final report has been agreed with Cheryl Miles) .

- The collection of biological material is progressing according to the planns approved by the US Army.

(10) REFERENCES:

None.

(11) APPENDICES:

None.

(13) FINAL REPORT:

To be due in December 2000.